

GROWTH OF HEMOLYTIC STREPTOCOCCI
AND INFLUENZA BACILLI IN PROTEIN FRACTIONS OF
BEEF BLOOD AND HUMAN ASCITIC FLUID

BY

WALTER RICHARD HESS

B.S., University of Nebraska, 1927

M.S., University of Illinois, 1937

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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ACKNOWLEDGMENTS

Acknowledgment is hereby given to the following persons for their aid, advice and constructive criticisms:

Dr. W. H. Welker, Head of the Department of Physiological Chemistry,
College of Medicine, University of Illinois.

Dr. Milan Novak, Department of Bacteriology, College of Medicine,
University of Illinois.

Dr. E. E. Rice, Research Laboratories, Swift and Company.

Irene Searson Hess, wife of author

INTRODUCTION

The growth of certain bacteria which are fastidious in their food requirements, such as hemolytic streptococci and influenza bacilli, is promoted by the addition of whole blood, blood serum or ascitic fluid to culture media. Because such organisms have lived in tissue or in blood for a long time, they have lost the power to synthesize some of the factors that are essential to their existence. Consequently, they must depend upon blood, tissue or some other outside source to supply growth factors that they no longer can synthesize. These factors are not only vitamins, but various protein constituents, as well.

The purpose of this study was to determine the growth-promoting properties of various protein fractions of beef blood and human ascitic fluid with respect to certain hemolytic streptococci and influenza bacilli. Consideration was also given to vitamins and enzyme systems which are essential for the growth of these organisms.

The protein fractions prepared for this work were albumin, pseudoglobulin, hemoglobin, serum dialysate, red cell dialysate, ascitic fluid dialysate, peptonized blood fibrin and peptonized casein. The last substance named was used in only one instance, for comparison. Most of the studies were centered around the dialysates, since they contained the small molecule protein and the vitamin systems, both of which were important for the growth of streptococci and influenza bacilli.

In blood and ascitic fluid the small molecule protein is normally adsorbed to the larger protein molecule. During dialysis it becomes separated from the large molecule and passes through the cellophane membrane until equilibrium is established. The small amount of protein which passes through the membrane can scarcely be detected by the biuret test, but is readily detected by the ninhydrin test. The serum and ascitic fluid dialysates became opalescent when heated, although there was no coagulation upon heating red cell dialysate.

Streptococcus hemolyticus epidemicus and *Hemophilus influenzae* were grown in culture media containing the dialysates, the large molecule proteins, peptones and beef extract. Tomato juice was used in some instances as another source of naturally occurring vitamins and enzymes. Both solid and liquid media were used.

RESULTS

Hemolytic streptococci grew quite well in red cell dialysate when combined with either serum dialysate or ascitic fluid dialysate. No dialysate by itself supported the growth of streptococci to any appreciable extent. Excellent growth of streptococci was obtained when dialysates of serum and cells, or ascitic fluid and cells, were added to plain nutrient media.

Dried serum dialysate was less effective in stimulating growth than undried dialysate, because of the changes that took place in the proteins and enzyme systems during the drying process.

The small molecule protein was precipitated from the ascitic fluid, serum and cell dialysates by alcohol and acetone in the presence of electrolytes. When the small molecule was redissolved and combined with the dialysate from which it was precipitated, the dialysate supported only a slight growth of streptococci. Denaturation of the protein and changes in the enzyme systems partially accounted for the lack of growth.

Difco peptone greatly improved the growth of hemolytic streptococci when added to serum or cell dialysate. Peptonized casein and peptonized beef blood fibrin improved the growth of streptococci in dialysate media only slightly. Beef extract improved the growth of streptococci in serum and ascitic fluid dialysates, but not in cell dialysate or in peptone water.

The addition of purified beef blood albumin, pseudoglobulin or hemoglobin to dialysate media did not increase the amount of growth of influenza bacilli, but did increase the amount of growth of hemolytic streptococci.

In all the experiments with hemolytic streptococci it was observed that growth did not take place unless either small molecule protein or peptone was present. The dialysates which contained the small molecule protein and the peptone solution also contained certain of the required vitamins and enzyme systems. Thus a utilizable form of nitrogen plus the required enzymes must be present before growth of certain strains of hemolytic streptococci will occur.

Three of the vitamins, namely, riboflavin, pantothenic acid and nicotinic acid, were determined in serum, cell and ascitic fluid dialysates, in Difco peptone, Difco beef extract, and in tomato juice. The results of the assays are shown in Table I.

TABLE I
Vitamin Assays in Gamma per C.C.

	Riboflavin	Pantothenic Acid	Nicotinic Acid
Serum Dialysate	0.004	0.045	0.21
Cell Dialysate	0.002	0.004	4.20
Ascitic Fluid Dialysate	0.015	0.066	0.18
2% Difco Peptone	0.005	0.120	0.85
2% Difco Beef Extract	0.40	2.00	10.00
Tomato Juice	0.20	0.41	4.15

The vitamins present in serum dialysate and ascitic fluid dialysate were very similar. Cell dialysate contained twenty times the amount of nicotinic acid as the serum or ascitic fluid dialysates, while the serum and ascitic fluid dialysates contained approximately ten times the quantity of pantothenic acid as did the red cell dialysate. The quantities of vitamins in each of the dialysates were reflected in the growth stimulation of hemolytic streptococci.

Beef blood red cell dialysate, alone, supported the growth of *Hemophilus influenzae* to a moderate extent. The addition of serum dialysate improved the growth considerably. Although red cell dialysate gave a negative benzidine test, it gave a positive peroxidase test. Peroxidase, a hemin compound, probably acted as the X-factor.

VITA

Walter Richard Hess was born in Elkhorn, Nebraska, December 8, 1903. He graduated from Elkhorn High School in 1921. He received his Bachelor of Science degree in chemistry in 1927 from the University of Nebraska, at Lincoln.

In 1933 he began work as a part time student toward his Master's degree in the University of Nebraska Medical School at Omaha. This work was interrupted in 1934 when he left Omaha to become a research bacteriologist in Chicago.

He resumed his studies in the University of Illinois in 1935, taking only part time work. He received his Master of Science degree in bacteriology from the University of Illinois in 1937.

His publications are as follows:

1. "The Mechanism of Gas Formation by the Genus *Bacillus* in Nitrate-Sugar-Meat Media." Master's Thesis.
2. "Fermentation in Meat Products by the Genus *Bacillus*," with Dr. L. B. Jensen. Food Res. 6:75, 1941.
3. "A Study of Ham Souring," with Dr. L. B. Jensen. Food Res. 6:273, 1941.
4. A Study of the Effects of Sodium Nitrate on Bacteria in Meat," with Dr. L. B. Jensen. Canner, Convention Number, 1941. Also Food Manufacture (London, England) 16:157, 1941.



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